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A Cometabolic Biotransformation Model for Halogenated Aliphatic Compounds Exhibiting Product Toxicity

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■ A model is proposed to describe the rate and extent of cometabolic transformation of halogenated aliphatic compounds by resting microbial cells. The finite transformation capacity (T_c) of resting cells, which appears to be associated with cometabolic oxidation of many halogenated aliphatic compounds, is used to incorporate the effects of product toxicity and reductant supply into a modified expression of Monod kinetics. Applicability of the model to trichloroethylene transformation by resting cells from a mixed methanotrophic culture is evaluated by comparison with experimental data from batch transformation studies conducted over a range of conditions. A visually good and statistically reasonable fit was obtained between the experimental data and model predictions both with cells alone and with formate added as an exogenous reductant source. A comparison of parameter estimates (k and K_s) derived by use of the cometabolic transformation model and those derived by use of conventional linearized Monod techniques (Lineweaver–Burk and concentration-normalized equations) indicates that, for reactions involving a finite transformation capacity, the linearized Monod equations yield artificially elevated parameters estimates.

Introduction

The widespread occurrence of water contaminated with halogenated aliphatic compounds such as the common solvent trichloroethylene (TCE) has led to development of treatment methods, including air stripping and activated carbon adsorption, for their removal. However, rather than causing complete contaminant destruction, these processes only transfer contaminants from one medium to another. In recent years, a variety of microbial processes have been discovered that can bring about the transformation and frequently the destruction of halogenated aliphatic compounds, stimulating increased interest in the potential of biological treatment. Unlike the biological processes commonly used for water or wastewater treatment, biological processes for the treatment of halogenated aliphatic compounds often depend upon cometabolism. Cometabolism is the transformation of a compound by organisms that do not obtain energy or carbon for cell growth from the transformation and hence require an alternative source of carbon and energy. There have been few engineering applications of cometabolism, especially for contaminant treatment, and the factors affecting the kinetics of cometabolism have not been studied extensively. Thus, principles upon which to base treatment process design are limited.

The unsuitability of basic Monod kinetics alone for application to methanotrophic TCE transformation reactions (*1*) hinders process design modeling attempts. However, recent work on cometabolic cofactor dependency, product toxicity, and competitive inhibition makes it possible to more adequately address the issues involved and to propose a model to describe cometabolic transformation rates and extents. This model and its experimental evaluation with data from batch transformation studies are presented in the following study. Throughout this paper the term "resting cells" refers to organisms in the absence of growth substrate (e.g., methanotrophs without methane).

Background

Halogenated aliphatic compounds were first discovered to be biologically transformed under reducing conditions where hydrogenolysis or dihaloelimination to a variety of more reduced products results (*2–4*), some of which are more hazardous than the parent compound (*5*). Wilson and Wilson (*6*) later reported on the possibility of aerobic oxidation of TCE by soil microorganisms that were provided natural gas as a primary source of energy. Here, methanotrophic bacteria were believed to transform TCE trough cometabolism, and this has now been adequately confirmed (*7–9*). The enzyme responsible is methane monooxygenase (MMO), which is used in the initial step of methane oxidation. Since then, other oxygenases have been found to be capable of TCE transformation, including those expressed during oxidation of toluene and other aromatic hydrocarbons (*10*, *11*), propane (*12*, *13*), and ammonia (*14*). Such oxidations require energy or reducing power, usually in the form of NADH or NADPH (*15*, *16*), and this must be available for TCE cometabolism as well (*9*).

Hou (*17*) explored the potential of methanotrophs for cometabolic epoxidation of propene for industrial use and demonstrated that propene oxidation continued for a period in the absence of methane. By supplying a pulse of methanol, an alternative substrate for methanotrophs that does not require MMO, the organisms were able to reestablish propene oxidation for an additional period. Hou

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attributed the ability of resting cells to carry out limited cometabolism to the presence of endogenous reducing power. Limited TCE oxidation by methanotrophic resting cells has also been demonstrated (*1*, *8*, *9*, *18–20*). Henry and Grbic-Galic (*20*) suggested the reducing power of resting methanotrophic cells is related to poly(hydroxybutyrate) (PHB) granule storage within the cells.

Alvarez-Cohen and McCarty (*1*) defined two terms related to the transformation ability of resting cells: *transformation capacity* (T_c), representing the maximum mass of cometabolized compound (contaminant) that can be transformed per unit mass of resting cells, and *transformation yield* (T_y), the maximum mass of cometabolized compound that can be transformed by resting cells per unit mass of primary substrate used for original cellular growth. For resting cells of a mixed methanotrophic culture grown on methane, they reported a T_c of 0.036 mg of TCE/mg of cells and a T_y of 0.013 mg of TCE/mg of CH_4.

Recent studies have indicated that T_c for cometabolic TCE transformation is not only a function of the availability of reducing power, but also of the specific cometabolized compound and toxicity of transformation products as well (*1*, *20*, *21*). Wackett and Householder clearly demonstrated with a pure culture of toluene-degrading organisms that the intermediate transformation products resulting from epoxidation of TCE were toxic to cells (*21*). Similar toxicity to methanotrophs has been reported by Alvarez-Cohen and McCarty (*1*) and Henry and Grbic-Galic (*20*). In the latter two cases, toxicity was indicated by the greatly reduced methane oxidation rate by the cultures after TCE transformation had occurred.

Studies have been conducted in order to maximize methanotrophic transformation rates of nongrowth substrates by examining the effects of temperature (*22–24*), cosubstrate addition (*1*, *9*, *20*, *23*), pH (*22*, *24*), and copper in the growth medium (*9*, *24*).

In the following study, a rate equation for the cometabolic transformation of halogenated aliphatics by resting cells is developed that incorporates the above noted finite transformation capacity. Experimental data are then provided to test the model applicability and to elucidate the important parameters for process design.

Model Development

Monod kinetics have often been used to relate the transformation rate of a compound to its concentration in solution (*25*):

$$-dS/dt = \frac{kXS}{K_s + S} \tag{1}$$

where S is the solution concentration of cometabolized contaminant (mg/L), k is the maximum rate of contaminant transformation (mg of S (mg of cells)$^{-1}$ day^{-1}), K_s is the half-velocity constant (mg/L), and X is the active microbial concentration (mg/L).

For the cometabolic transformation of a contaminant by resting cells, there is no microbial growth over the course of the transformation. Microbial reactions not supporting growth are most commonly modeled by Monod kinetics with a constant active microbial concentration (*26*, *27*), but have also been modeled by Monod kinetics with an active microbial concentration that decays over time (*28*), or with incorporation of competitive product inhibition (*29*), as well as by first-order kinetics (*9*, *19*). However, for the cometabolic oxidation of halogenated aliphatic compounds, product toxicity results in a finite transformation capacity of resting cells. Hence, the overall activity of resting cells appears to decrease in proportion to the amount of cometabolized contaminant consumed. On this basis, the transformation capacity of resting cells might be expressed in the form

$$\frac{1}{T_c} = \frac{dX}{dS} = \frac{\text{mass of cells inactivated}}{\text{mass of contaminant transformed}} \tag{2}$$

which when integrated yields the following expression for the active microbial concentration introduced previously (*30*):

$$X = X_0 - \frac{1}{T_c}(S_0 - S) \tag{3}$$

where X_0 is the initial active microbial concentration (mg/L), X is the active microbial concentration at time t (mg/L), S_0 is the initial concentration of cometabolized contaminant (mg/L), and S is the concentration of cometabolized contaminant at time t (mg/L).

Combining eqs 1 and 3 and rearranging gives the following:

$$dt = \frac{-(K_s + S)}{k\left(X_0 - \frac{1}{T_c}(S_0 - S)\right)S}\,dS \tag{4}$$

which can be integrated over time for a batch reactor to yield the following relationship between S and t:

$$t = \frac{1}{k}\left[\left(\frac{K_s}{S_0/T_c - X_0}\right)\ln\left\langle\frac{SX_0}{\left[X_0 - \frac{1}{T_c}(S_0 - S)\right]S_0}\right\rangle + T_c \ln\left\langle\frac{X_0}{\left[X_0 - \frac{1}{T_c}(S_0 - S)\right]}\right\rangle\right] \tag{5}$$

Equation 5 relates the cometabolized contaminant concentration remaining at any time t to the initial contaminant and organism concentration for a given transformation capacity. Two notable aspects of eq 5 are the following: (1) $S_0/T_c - X_0$ in the denominator renders the solution discontinuous when $S_0/T_c = X_0$, implying that the complete utilization of transformation capacity will not occur, and (2) real solutions can only be obtained when $X_0T_c > (S_0 - S)$, indicating that the extent of transformation cannot exceed the transformation capacity. Results of the following experimental study were used to evaluate the suitability of this equation for prediction of TCE transformation by a mixed methanotrophic culture and to evaluate values for the terms k and K_s.

Materials and Methods

Mixed-Culture Development. A 7.5-L stirred tank microbial growth reactor was seeded with effluent from a laboratory column of aquifer material that had been enriched with methane and oxygen as previously described (*1*). The reactor was operated at a 9-day hydraulic detention time by addition of 833 mL/day of medium and once daily cell wasting. The growth medium consisted of mineral salts dissolved in deionized water after that of Fogel et al. (*31*). A mixture of 10.3% methane in air was continually injected into the reactor bottom at 280 mL/min and 1.035 atm partial pressure; high-velocity mixing (200 rpm) was maintained to facilitate methane and oxygen transfer to the liquid phase. The measured liquid concentrations of 0.02 mg/L methane and 3.5 mg/L oxygen indicated that cell growth was methane limited. A net growth yield of 0.33–0.37 g of cells/g of CH_4 consumed was

Table I. Parameter Values for the Nonlinear Least-Squares Fit of the Cometabolic Transformation Model to Formate-Free Methanotrophic TCE Disappearance Data (Experiment A)

parameter	value	asymptotic SE	95% confid interv	corr matrix		
k, mg of TCE (mg of cells)$^{-1}$ day^{-1}	0.53	0.017	0.48–0.57	1		
K_s, mg/L	0.37	0.098	0.13–0.61	0.857	1	
S_0, mg/L	14.7	16.3	14.3–15.1	0.788	0.458	1
r^2 = 0.9995						

indicated by the average cell density of 2500 mg/L (ranging from 1800 to 3000 mg/L) and the gas effluent of 8.6% methane.

TCE Solutions and Analyses. Water-saturated TCE solution was prepared at least 24 h before use by adding 10 mL of TCE (99+% pure ACS reagent, Aldrich Chemicals Co., Milwaukee, WI) to a 160-mL glass bottle containing five glass beads and 120 mL of Milli-Q water. The bottle was sealed with a Teflon-lined rubber septum and aluminum crimp-top cap and vigorously shaken. One hour prior to use, the bottle was again shaken and allowed to settle. TCE-saturated water was removed by syringe through the septum, using care to exclude nonaqueous phase TCE.

TCE gaseous concentration was determined from headspace analysis as described previously (*1*), with a Tracor MT-220 gas chromatograph equipped with a packed column (10% squalence on Chromosorb A/AW) maintained at 70 °C and a linearized electron capture detector, and using an argon/methane mixture as carrier gas. A dimensionless Henry's constant of 0.31 for TCE at 21 °C (*32*) was used along with known liquid and gas volumes to compute TCE liquid concentrations and total TCE mass present.

TCE Transformation Studies. Transformation experiments were performed in a 21 °C environmental chamber using 62-mL glass bottles sealed with either Mininert Teflon-lined caps or a set of two 50-mil Teflon-lined septa (*1*). The bottles were inoculated with 20 mL of liquid (mixed-culture medium, cells, or a combination of both). For formate-supplied bottles, 1 mL of mixed-culture medium was replaced with 1 mL of a 400 mM sodium formate solution in Milli-Q water to yield a final concentration of 20 mM formate. Corresponding resting cells received 1 mL of pure Milli-Q water. Saturated TCE solution was added by gas-tight syringe through the Mininert valves, and the bottles were vigorously shaken by hand for 15 s before initial headspace samples for TCE were taken. The bottles were then shaken at 400 rpm (unless otherwise noted) on a circular action shaker table (Lab-Line). Gas samples (200 μL) were withdrawn periodically with a 500-μL gas-tight syringe (Pressure-lok) and 22-gauge side-port needle for TCE analysis. TCE transformation rates were determined from changes in total TCE mass, including both that in the liquid and in the gas phases.

Culture Density. Culture density was determined gravimetrically by adding a specific volume of suspended culture to tared 5.1-cm aluminum foil dishes and evaporating the dishes overnight at 105 °C before cooling and reweighing them. Medium controls were used to correct for inorganic dissolved solids in the culture medium. Concentrations reported represent total dry weight of the mixed culture in milligrams per liter.

Results

In experiment A with TCE alone (no formate addition), T_c was estimated by repeated addition of 15 mg of TCE/L to bottles containing 2300 mg/L resting cells (46 mg/bottle) until TCE transformation no longer occurred. From the results illustrated in Figure 1 (open box data) 1.7 mg of TCE (solution plus headspace mass) was transformed, indicating T_c equaled 0.036 mg of TCE/mg of cells. Next, S_0, k, and K_s were estimated by a nonlinear regression fit of eq 5 to the disappearance data from only the first TCE addition at the 14.5 mg of TCE/L level. Estimation of S_0 is necessitated by the inherent inaccuracy of a time zero headspace measurement of the volatile TCE due to short-term nonequilibrium between the headspace and liquid phase. Nonlinear regression analysis was performed using Systat 5.0 application software (Systat, Inc.) employing a quasi-Newton estimation method. This analysis resulted in k = 0.53 mg of TCE (mg of cells)$^{-1}$ day^{-1} and K_s = 0.37 mg/L (r^2 = 0.9995) with additional statistics summarized in Table I. These values for k, K_s, and T_c were introduced into eq 5, along with appropriate S_0 values, to examine the equation fit for the disappearance of the repeated TCE additions for this case. The results are shown plotted together with the experimental data in Figure 1 and summarized in Table II. In order to test the broader applicability of the model, the same parameters were applied under conditions of more dilute cell con-

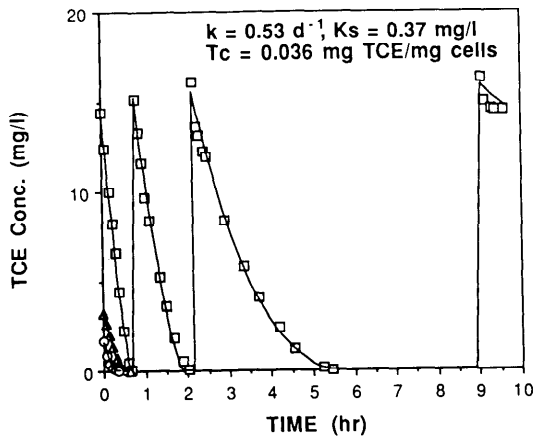

Figure 1. TCE disappearance in batch bottles at three initial TCE concentrations (experiment A). Experimental data (symbols) are plotted along with predictions (lines) by use of the cometabolic transformation model and k and K_s determined from nonlinear regression analysis of initial injection of 14.7 mg/L.

Table II. Residual Sum of Squares (RSS) and Initial TCE Concentrations for Fits of the Cometabolic Transformation Model[a]

	S_0, mg/L	no. of data points	RSS
first TCE addn[b]	14.7	9	0.206
second TCE addn	15.3	10	2.13
third TCE addn	15.6	12	2.75
fourth TCE addn	15.9	5	1.23
dilute cells	3.3	9	0.0624
dilute cells	1.7	6	0.0439

[a] Parameters estimated from the first TCE addition to formate-free methanotrophic cells (experiment A). [b] Data used for parameter estimation.

Table III. Parameter Values for the Nonlinear Least-Squares Fit of the Cometabolic Transformation Model to Formate-Amended Methanotrophic TCE Disappearance Data (Experiment B)

parameter	value	asymptotic SE	95% confid inter	corr matrix		
k, mg of TCE (mg of cells)$^{-1}$ day^{-1}	7.6	0.438	6.3–8.8	1		
K_s, mg/L	8.2	0.526	6.7–9.6	0.960	1	
S_0, mg/L	10.1	0.141	9.8–10.5	0.721	0.617	1
r^2 = 0.9984						

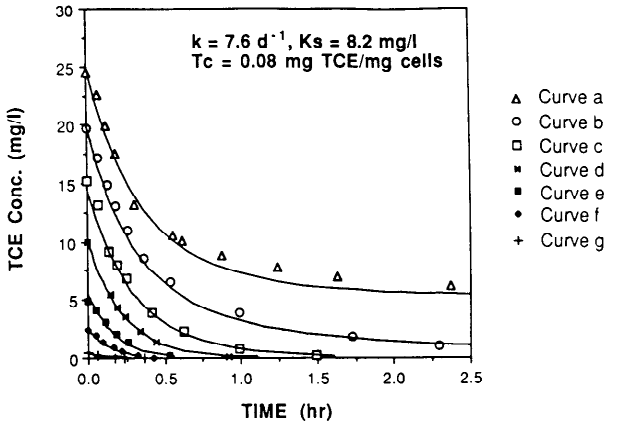

Figure 2. TCE disappearance in formate-amended batch bottles at seven initial TCE concentrations (experiment B). Experimental data (symbols) are plotted along with predictions (lines) by use of the cometabolic transformation model and k and K_s determined from nonlinear regression analysis of curve d.

centration (805 mg/L) and lower initial TCE concentrations (3.2 and 1.6 mg/L), with results also shown in Figure 1 and Table II. The fits between the data and the equation are good, as evidenced by the consistently low residual sum of squares for the repeated TCE additions as well as for the more dilute cell and TCE conditions.

Data from five additional similar experiments, which were fitted with eq 5, yielded the following parameter averages and standard deviations: T_c = 0.043 (0.010), k = 0.84 (0.29), K_s = 0.69 (0.54). The variations here are larger than expected from experimental errors alone, indicating that the model parameters may vary somewhat with change in operational characteristics of the culture. While the transformation capacity of resting cells freshly harvested from the reactor generally showed small variation, caution should be exercised in extending the values to other cultures or operational conditions.

Formate can be used as an external source of reducing power by methanotrophic resting cells (*33*). In order to determine how the addition of such a nongrowth-inducing and noncompetitive external source of reducing power would affect model parameters, bottles containing 421 mg/L (8.4 mg/bottle) resting cells and 20 mM NaCOOH were supplemented with seven different TCE concentrations in experiment B (Figure 2). The transformation capacity of the cells supplied with formate was determined from the mass of TCE consumed prior to the time that transformation ceased in the bottle initially amended with 25 mg/L TCE (curve a), where TCE utilization was not complete. The value found (T_c = 0.080 mg of TCE/mg of cells) was over twice that found with TCE alone, indicating that the addition of reducing power was here highly beneficial for increasing the transformation capacity. The values of S_0, k, and K_s were obtained from the nonlinear regression fit of the disappearance data from the bottle receiving the middle concentration (10 mg/L) of the range of TCE additions (curve d). The resultant parameter estimates were k = 7.6 mg of TCE (mg of cells)$^{-1}$ day^{-1} and K_s = 8.2 mg/L (r^2 = 0.9984), with the additional statistics

Table IV. Residual Sum of Squares (RSS) and Initial TCE Concentrations for Fits of the Cometabolic Transformation Model[a]

curve	S_0, mg/L	no. of data points	RSS
a	25.2	12	7.11
b	19.9	13	2.42
c	14.6	9	2.62
d[b]	10.1	7	0.021
e	5.7	6	0.598
f	2.6	7	0.114
g	0.36	5	0.0135

[a] Parameters estimated from curve d of formate amended methanotrophic TCE disappearance data (experiment B). [b] Data used for parameter estimation.

summarized in Table III. The fit between the TCE disappearance using eq 5 and experimental data from the seven different TCE levels is again good (Figure 2, Table IV), indicating the model is applicable over a wide range of initial TCE concentrations. The estimates of both k and K_s along with their respective 95% confidence intervals are higher with formate added than in the absence of formate, although the ratio k/K_s with formate is somewhat lower (1.4 vs 0.93 L mg^{-1} day^{-1}). Combining results from three additional similar experiments yielded parameter averages and standard deviations of T_c = 0.061 (0.025), k = 4.8 (1.9), and K_s = 7.9 (0.62). However, the high correlation between k and K_s for experiment B (Table III) indicates that when formate is added to resting cells within the TCE concentration range studied, it may not be possible to obtain unique parameter estimates of k and K_s, suggesting that for those experimental conditions the k/K_s ratio may be a more useful kinetic parameter.

In order to further evaluate the uniqueness of parameter estimates and elucidate the relative importance of parameters over the specific concentration ranges of interest, a sensitivity analysis such as that described by Robinson and Characklis (*34*, *35*) was performed. The sensitivity equations were derived from eq 5 by taking the first derivative of the dependent variable with respect to the parameter of interest (dS/dS_0, dS/dk, dS/dK_s) by implicit differentiation. The resultant equations were multiplied by their respective parameters to yield consistant units (mg of TCE/L) and are shown plotted against the dependent variable (S) for experiment A in Figure 3 and experiment B in Figure 4. The lack of proportionality between all three curves for both initial concentrations in experiment A (Figure 3) suggests that those experimental conditions should yield unique parameter estimates over most TCE concentrations. However, the relatively low value of the K_s equation at both initial concentrations (note the equation is multiplied by 10 in Figure 3) suggests that, for the conditions of experiment A, the model is relatively insensitive to changes in K_s. For the conditions of experiment B, the sensitivity equations in Figure 4 show a high proportionality of the k and K_s equations for all three initial concentrations, suggesting here that unique estimates of both k and K_s may not have been obtained.

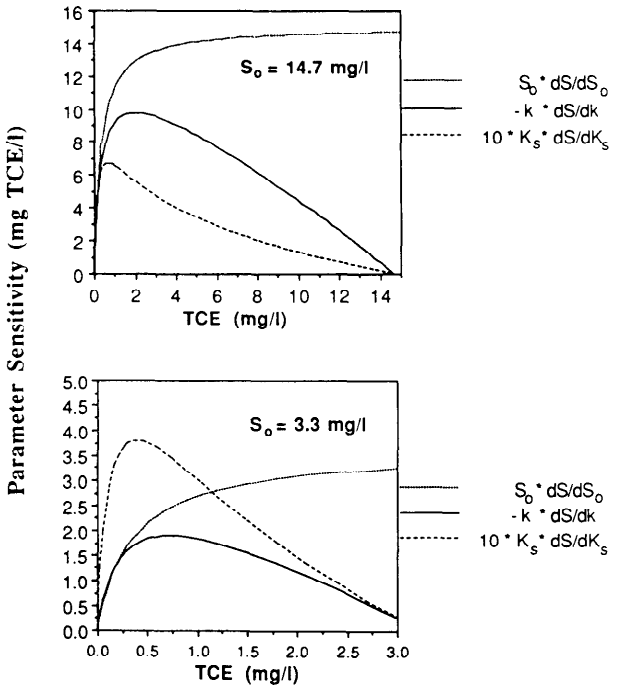

Figure 3. Sensitivity equations for parameters of the cometabolic transformation model applied to methanotrophic TCE transformation under conditions of experiment A for two initial TCE concentrations.

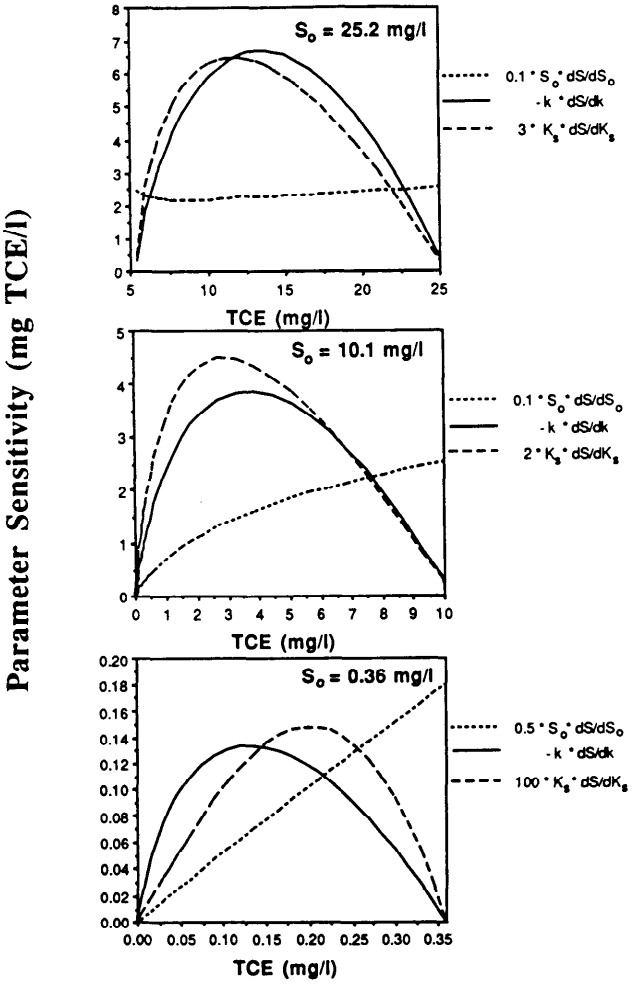

Figure 4. Sensitivity equations of parameters of the cometabolic transformation model applied to methanotrophic TCE transformation under conditions of experiment B for three intial TCE concentrations.

Additionally, Figure 4 shows that at the low initial TCE concentration (0.36 mg/L) the model is extremely insensitive to K_s while at the higher initial concentrations much more sensitivity is observed.

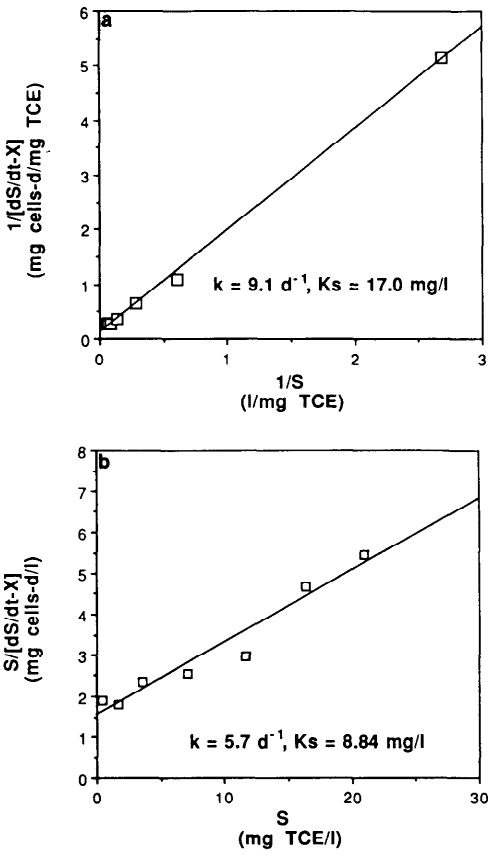

Figure 5. (a) Lineweaver–Burk plot of rate of TCE disappearance within the initial 12 min in formate-fed batch bottles at seven initial TCE concentrations. (b) Concentration-normalized plot of rate of TCE disappearance within the initial 12 min in formate-fed batch bottles at seven initial TCE concentrations.

Table V. Kinetic Parameters for Formate-Amended Methanotrophic TCE Transformation Computed by Three Different Methods

calculation method	k, mg of TCE (mg of cells)$^{-1}$ day^{-1}	K_s, mg of TCE/L	k/K_s, L (mg of cells)$^{-1}$ day^{-1}	r^2
Lineweaver–Burk	9.1	17.0	0.54	0.998
concentration-normalized	5.7	8.8	0.65	0.943
nonlinear cometabolic transformation model	7.6	8.2	0.93	0.998

In order to compare results obtained with the cometabolic transformation model (eq 5) and more conventional techniques, values for k and K_s were estimated from a Lineweaver–Burk plot (Figure 5a) and a concentration-normalized plot (Figure 5b) of initial disappearance rates computed by linear regression analysis of experiment B data taken within the first 12 min after TCE addition for each TCE concentration, a time period over which cell inactivation might be expected to be small. The parameters derived by these two methods as well as those computed by using nonlinear regression fit of eq 5 to the experimental data are listed in Table V. The parameters computed by use of the Lineweaver–Burk approach are both significantly greater than when eq 5 is used, but are similar when the concentration-normalized method is used. However, both conventional methods produced lower k/K_s

ratios. Based upon the T_c value of 0.080 mg of TCE/mg of cells, it was calculated that the amount of transformation capacity consumed during the first 12 min ranged from 1.8% for the lowest concentration to 35% for the three highest concentrations. This suggests that use of eq 1 alone (basis of both the Lineweaver–Burk and concentration-normalized inverse plots) results in biased parameter estimations, especially at higher contaminant concentrations.

The higher values of K_s obtained by the two traditional methods are artificially elevated as a result of the falloff of the transformation rate associated with toxicity and/or electron donor supply. Consequently, the inflated K_s is accompanied by an artificially elevated k in the Lineweaver–Burk expression since use of the reciprocal plot exaggerates the weighting of the data at the lowest concentrations, where the transformation rate is a function of k/K_s. Conversely, in the normalized-concentration expression the effects of an elevated K_s are less predictable and directly dependent on the range of data since normalization causes the data to be more evenly weighted.

Discussion

The proposed model, which uses TCE transformation capacity to incorporate the effects of toxicity and electron donor supply into a modified expression of Monod kinetics, was capable of predicting the results of methanotrophic TCE transformation reactions over a range of conditions. As yet, the broader applicability of this model to other cometabolic reactions has not been tested. However, evidence of cometabolic finite transformation capacities of resting cells has been noted for many different bacterial systems, including methanogenic reductive dehalogenation reactions (*36*), TCE oxidation by toluene oxidizers (*10*) and ammonia oxidizers (*14*), and methanotrophic propene oxidations (*17*). A model of the type developed here may be applicable to this broad range of cometabolic reactions; however, further evaluation is needed.

The proposed cometabolic transformation model may also apply to circumstances in which a finite transformation capacity occurs in the absence of overt product toxicity, possibily caused by such phenomena as cofactor dependence, unstable enzymes, and starvation strategies. However, the proposed model by itself would not be applicable for the transformation of compounds that are themselves toxic, since the model presumes that cell activity falls off not as a function of the contaminant itself, but rather as a function of the amount of contaminant consumed. The ability of this model to successfully fit the data for TCE transformation by the methanotrophic culture studied lends further evidence to the hypothesis that, for this culture at least, it is the transformation products, not TCE itself, that are responsible for the observed cell toxicity.

Formate addition to resting methanotrophic cells has been shown to result in an increased maximum TCE transformation rate (*1*, *9*, *24*) and capacity (*1*, *9*). The increased maximum rate implies that the reducing power provided by formate may be more readily available than that from the cell's own internal energy reserves. Also, the increased transformation capacity with formate addition suggests that the internal reductant supply may indeed be somewhat limited. Nevertheless, it is still unclear exactly how product toxicity and reductant supply affect transformation capacity since the increased transformation rate caused by formate addition should result in an increased rate of transformation product appearance, and hence increased toxicity. Since the presence of formate enables a given mass of cells to transform a higher mass of substrate before inactivation, a product toxicity saturation is suggested. That is, the toxic effect is rate limited by some unknown factor such as transport into or within the cell or toxicity reaction kinetics. However, further studies are needed to clarify this issue.

Although it has been previously reported that aeration of methanotrophic resting cells results in decreased transformation rates (*1*), this effect has not been incorporated into the cometabolic transformation model proposed, since compared to the effect of TCE product toxicity on resting cells at the level studied, the aeration effect was an order of magnitude lower and thus of little importance here. Under other circumstanes, inclusion of a term for the decrease due to aeration alone may be appropriate.

The results of this study suggest that individual parameter values may not always be a good indicator of intrinsically higher transformation rates. Therefore, caution should be used when reported parameter values are interpreted, and when possible, experiments designed to directly measure maximum transformation rates at concentrations well above the K_s range.

The proposed cometabolic transformation model, which incorporates the effects of product toxicity and reductant supply into Monod kinetics, was shown to be applicable to methanotrophic TCE transformation by resting cells. A model of this type should be useful for evaluating various reactor designs and configurations for the cometabolic transformation of contaminants by resting microbial cells.

Registry No. TCE, 79-01-6; formate, 71-47-6.

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Two-Stage Dispersed-Growth Treatment of Halogenated Aliphatic Compounds by Cometabolism

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■ A two-stage bioreactor that utilizes cometabolic biotransformations for the treatment of halogenated aliphatics is proposed. Methanotrophic cells are grown in a dispersed-growth reactor prior to transferral to a plug flow transformation reactor in which they are contacted with the waste stream and transformation occurs. A model describing cometabolic biotransformation is used together with basic equations for design of the growth and treatment reactors to predict treatment efficiencies and to evaluate the effects of the finite transformation capacity of resting cells, electron donor supply, and product toxicity on process design. For an example treatment scenario targeting trichloroethylene (TCE), methane transfer and growth reactor size are found to dominate the system design at high contaminant concentrations, while at low concentrations, the treatment reactor size becomes more important. The results of this analysis for a two-stage suspended-growth reactor system suggest that increasing methane and oxygen mass-transfer rates, cell yield, and transformation capacity may have a greater impact on reducing overall reactor size than would an increase in trichloroethylene transformation rate.

Introduction

The growing use of halogenated aliphatic compounds and their subsequent release into the environment indicates the need for the development of a low-cost, highly effective treatment system for their destruction. At present, the most prevalently used treatment processes for halogenated organics include air stripping and activated carbon sorption, which are capable of purifying water and gas streams, but simply transfer the organic contaminants to a new phase without destroying them.

Many halogenated compounds such as trichloroethylene (TCE) have not been shown to be used by bacteria for energy or growth, but can be transformed through cometabolism by organisms that use a primary substrate, such as methane, for metabolism (*1-4*). A treatment system based upon the cometabolic transformation of halogenated aliphatics by methanotrophic microorganisms may be a cost-effective and efficient alternative to physical processes due to its potential for high transformation rates, complete compound degradation without formation of undesirable end products, applicability to a broad range of compounds, and a requirement for an inexpensive and widely available primary growth substrate.

Since methanotrophic TCE and methane oxidation both require the same key enzyme, competitive inhibition significantly affects the cometabolic transformation kinetics, as evidenced both in suspended-growth (*5*) and unsaturated fixed-film bioreactors (*6*). Competitive inhibition must therefore be factored into process design. Previous studies with methanotrophic bioreactors have used single-stage reactors in which competitive inhibition makes optimization of transformation efficiency difficult (*6-9*).

However, methanotrophic cells are capable of transforming TCE in the absence of methane (resting cells), and in this way, competitive inhibition can be avoided. A recent finding of significance is that product toxicity and limited electron donor supply result in a finite *transformation capacity* (T_c) of resting cells (*10*). Here, T_c is defined as the maximum mass of TCE transformed by a unit mass of resting cells (mg of TCE/mg of cells); a corresponding term, the *transformation yield* (T_y), represents the maximum mass of TCE transformation per mass of CH_4 used to grow the cells (mg of TCE/mg of CH_4). Formate addition can significantly increase T_c and T_y, presumably due to the increased supply of electron

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